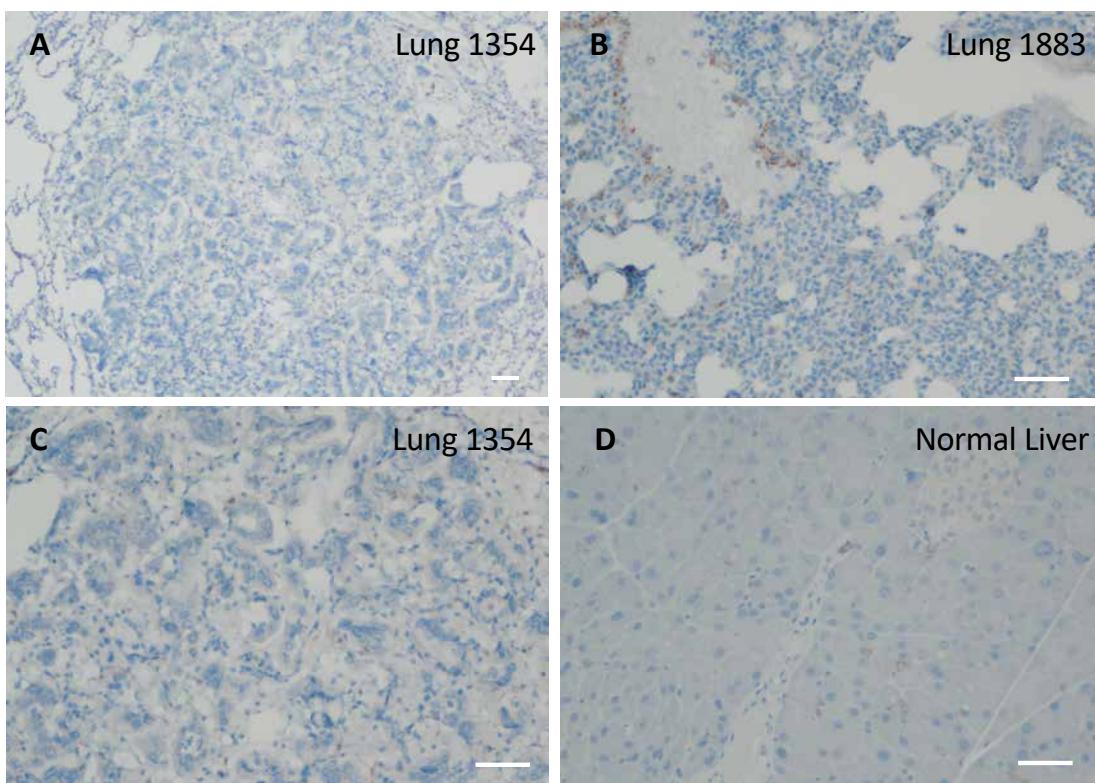


Supplementary Fig. 1 – supports Fig. 1

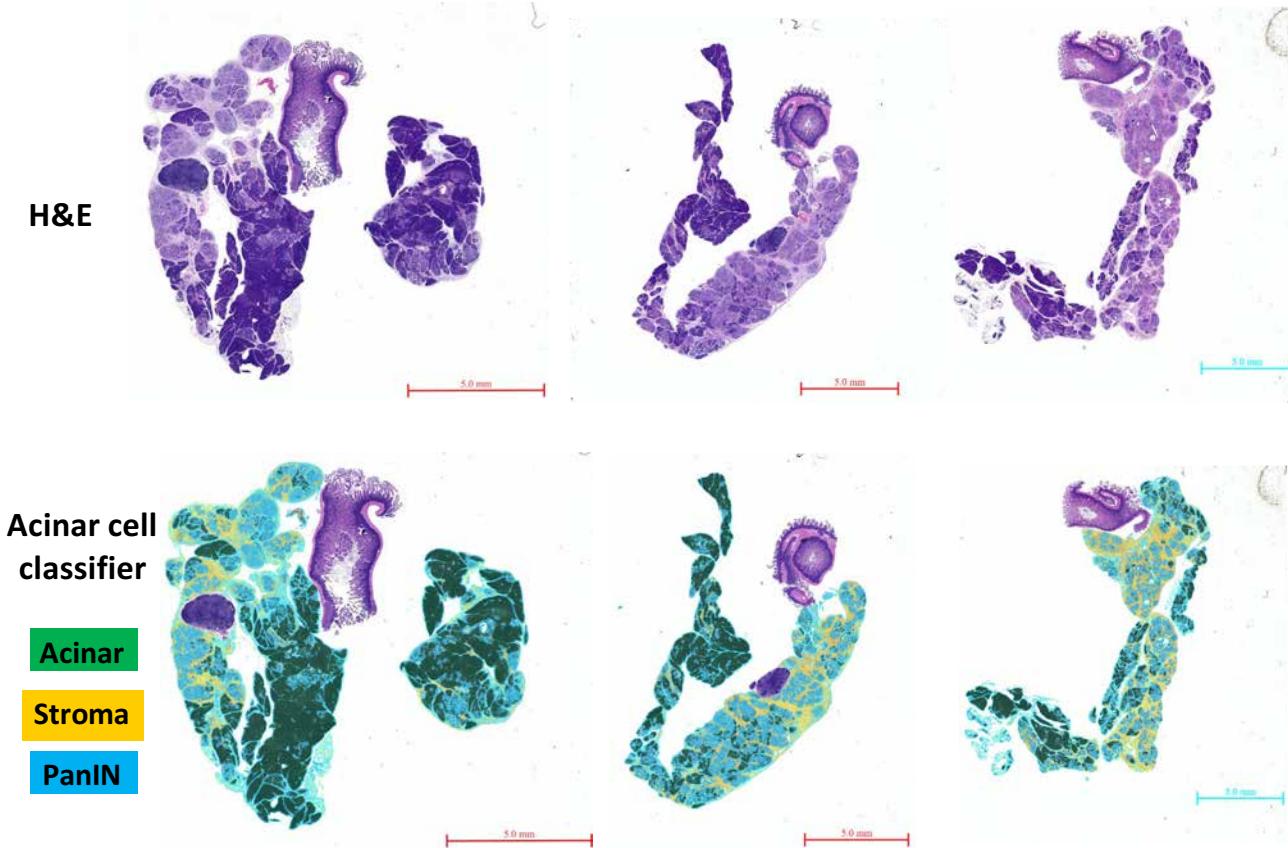
ID	Genotype	age (days)	Macroscopic Metastasis	Macroscopic Cyst	PDA by IHC	Met phenotype	Met location
1216	K ⁺ ; p48Cre; WT/WT	323	N	N	Y	NA	
1416	K ⁺ ; p48Cre; WT/WT	275	N	N	Y	NA	
1985	K ⁺ ; p48Cre; WT/WT	629	N	N	Y	NA	
2033	K ⁺ ; p48Cre; WT/WT	703	N	N	Y	NA	
2633	K ⁺ ; p48Cre; WT/WT	399	N	N	Y	NA	
2687	K ⁺ ; p48Cre; WT/WT	447	N	N	Y	NA	
4750	K ⁺ ; p48Cre; WT/WT	117	N	N	Y	NA	
1534	K ⁺ ; p48Cre; WT/WT	306	NO INFO	NO INFO	NO INFO	NA	
1741	K ⁺ ; p48Cre; WT/WT	382	NO INFO	NO INFO	NO INFO	NA	
1744	K ⁺ ; p48Cre; WT/WT	382	NO INFO	NO INFO	NO INFO	NA	
4067	K ⁺ ; p48Cre; WT/WT	340	NO INFO	NO INFO	NO INFO	NA	
4312	K ⁺ ; p48Cre; WT/WT	700	Y	Y	Y	PDA	Liver
1220	K ⁺ ; p48Cre; WT/WT	222	Y	N	Y	PDA	Liver
1354	K ⁺ ; p48Cre; WT/WT	370	Y	N	Y	PDA	Liver, Lung, Diaphragm
1356	K ⁺ ; p48Cre; WT/WT	370	Y	N	Y	PDA	Diaphragm
1414	K ⁺ ; p48Cre; WT/WT	262	Y	N	Y	PDA	Liver
1415	K ⁺ ; p48Cre; WT/WT	232	Y	N	Y	PDA	Liver
1588	K ⁺ ; p48Cre; WT/WT	443	Y	N	Y	PDA	Liver
1692	K ⁺ ; p48Cre; WT/WT	305	Y	N	N	Osteosarcoma	
1883	K ⁺ ; p48Cre; WT/WT	403	Y	N	Y	PDA	Liver, Lung, Inv to kidney
2031	K ⁺ ; p48Cre; WT/WT	521	Y	Y	Y	PDA	Liver, Lymph nodes
2032	K ⁺ ; p48Cre; WT/WT	530	Y	Y	N	Hematopoietic neoplasia	
2137	K ⁺ ; p48Cre; WT/WT	316	Y	N	Y	PDA	Liver
283	K ⁺ ; p48Cre; CK1/CK1	484	N	Y	Y	NA	
953	K ⁺ ; p48Cre; CK1/CK1	722	N	N	Y	NA	
1042	K ⁺ ; p48Cre; CK1/CK1	214	N	N	N	NA	
1068	K ⁺ ; p48Cre; CK1/CK1	722	N	Y	Y	NA	
1099	K ⁺ ; p48Cre; CK1/CK1	841	N	N	Y	NA	
1128	K ⁺ ; p48Cre; CK1/CK1	957	N	Y	Y	NA	
1130	K ⁺ ; p48Cre; CK1/CK1	653	N	N	Y	NA	
1135	K ⁺ ; p48Cre; CK1/CK1	486	N	Y	Y	NA	
1139	K ⁺ ; p48Cre; CK1/CK1	697	N	Y	Y	NA	
1264	K ⁺ ; p48Cre; CK1/CK1	412	N	N	Y	NA	
1269	K ⁺ ; p48Cre; CK1/CK1	147	N	N	Y	NA	
1592	K ⁺ ; p48Cre; CK1/CK1	509	N	N	Y	NA	
1995	K ⁺ ; p48Cre; CK1/CK1	565	N	N	Y	NA	
3113	K ⁺ ; p48Cre; CK1/CK1	364	N	Y	Y	NA	
4739	K ⁺ ; p48Cre; CK1/CK1	351	N	N	Y	NA	
508	K ⁺ ; p48Cre; CK1/CK1	180	NO INFO	NO INFO	NO INFO	NO INFO	
965	K ⁺ ; p48Cre; CK1/CK1	584	NO INFO	NO INFO	NO INFO	NO INFO	
1271	K ⁺ ; p48Cre; CK1/CK1	505	NO INFO	NO INFO	NO INFO	NO INFO	
1308	K ⁺ ; p48Cre; CK1/CK1	415	NO INFO	NO INFO	NO INFO	NO INFO	
1309	K ⁺ ; p48Cre; CK1/CK1	552	NO INFO	NO INFO	NO INFO	NO INFO	
744	K ⁺ ; p48Cre; CK1/CK1	292	Y	N	Y	PDA	Liver
809	K ⁺ ; p48Cre; CK1/CK1	697	Y	Y	Y	Hematopoietic neoplasia	
963	K ⁺ ; p48Cre; CK1/CK1	584	Y	N	Y	PDA	Liver
1044	K ⁺ ; p48Cre; CK1/CK1	837	Y	Y	Y	Sarcoma	
1131	K ⁺ ; p48Cre; CK1/CK1	379	Y	N	Y	PDA	Liver
1382	K ⁺ ; p48Cre; CK1/CK1	310	Y	N	Y	PDA	Liver, Lung
1583	K ⁺ ; p48Cre; CK1/CK1	326	Y	N	Y	PDA	Liver
1942	K ⁺ ; p48Cre; CK1/CK1	126	Y	Y	Y	Kidney	
3639	K ⁺ ; p48Cre; CK1/CK1	270	Y	N	Y	hematopoietic neoplasia	



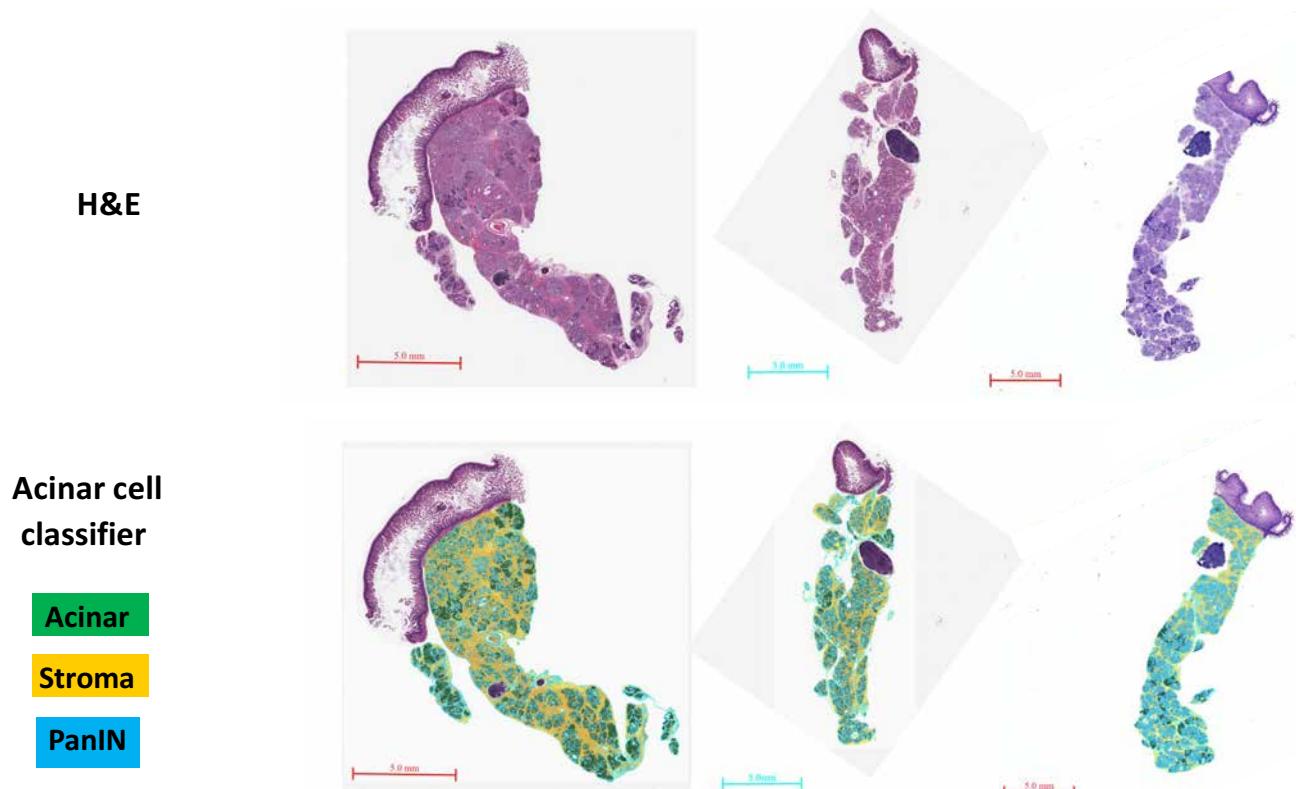
Examples of Cx43 staining in lung metastases (A-C) and normal liver (D). Mag bars are 50μm.

Supplemental Fig. 2
Supports Fig. 1

Examples of KC pancreata from 6 month old mice



Examples of KC^{CK1A} pancreata from 6 month old mice

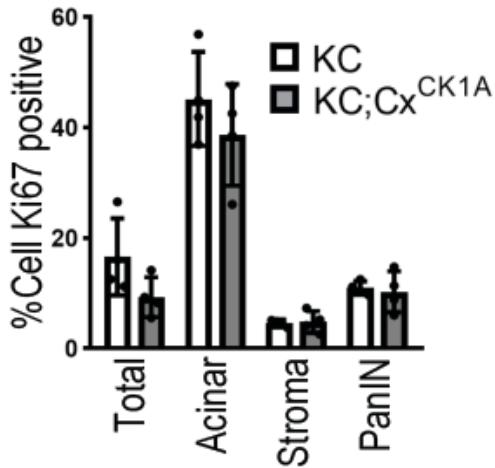


Low magnification representative H&E images of pancreata from 6-month old animals. A classifier was developed to distinguish acinar (green), stromal (yellow) and PanIN (blue) compartments.

Supplemental Fig. 3

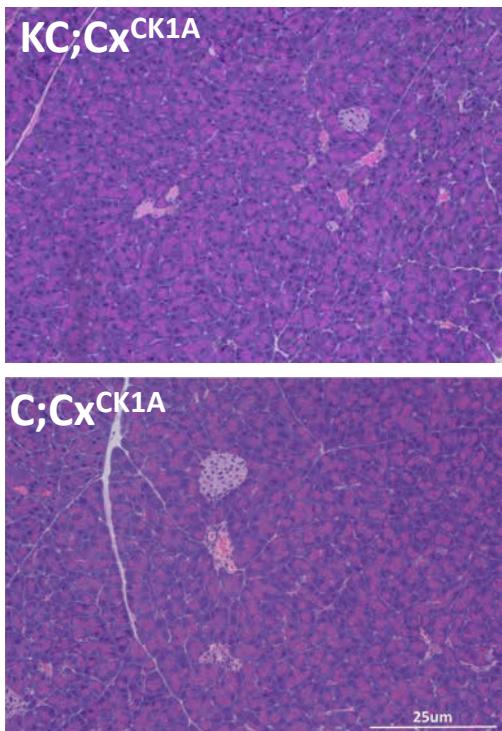
Supports Fig. 2

Ki67 analysis 6-month tumors



Ki67 staining was quantified in pancreata from 6-month old mice (n=4/genotype). Using a classifier to distinguish acinar, stromal and PanIN compartments we saw no difference in proliferation between the genotypes.

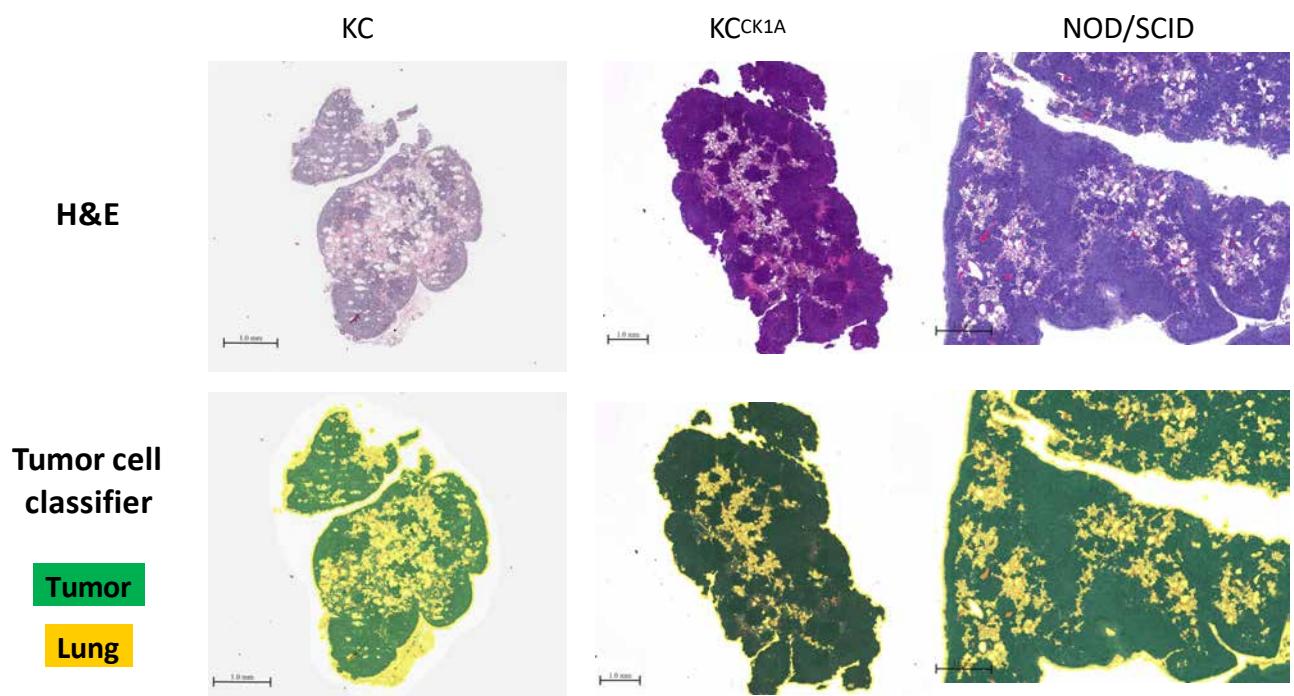
Pancreas H & E stained



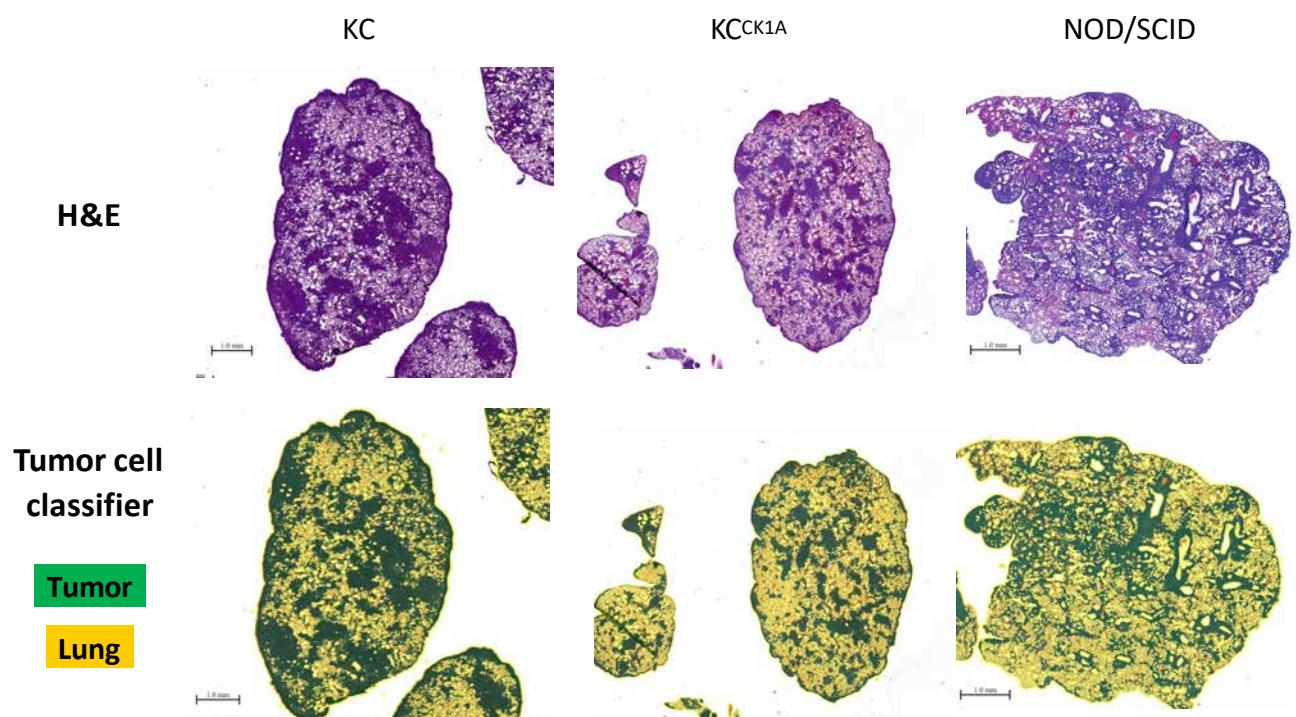
H&E staining of pancreas from 3 month-old mice.

Supplemental Fig. 4
Supports Fig. 3

Representative examples of lung from KC^{CK1A} donor cell tail vein experiments

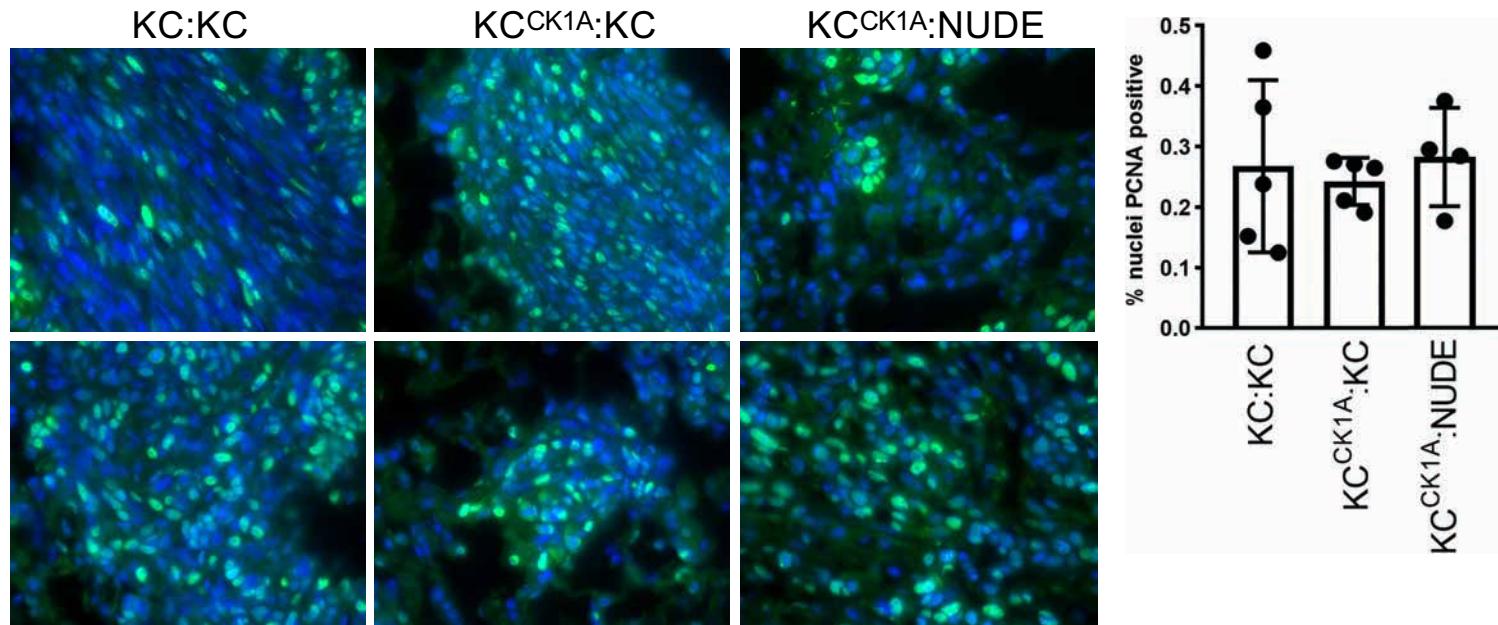


Representative examples of lung from KC donor cell tail vein experiments



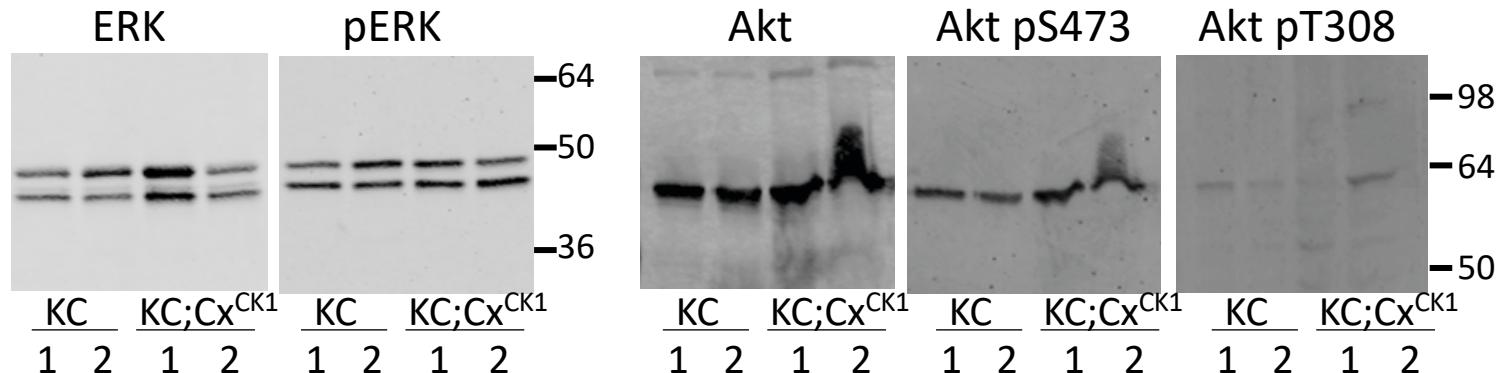
Low magnification representative H&E images of mouse lungs harvested 2 weeks after tail vein injections. A classifier was developed to distinguish normal lung tissue (yellow) from tumor cells (green).

Supplemental Fig. 5 supports Fig. 3



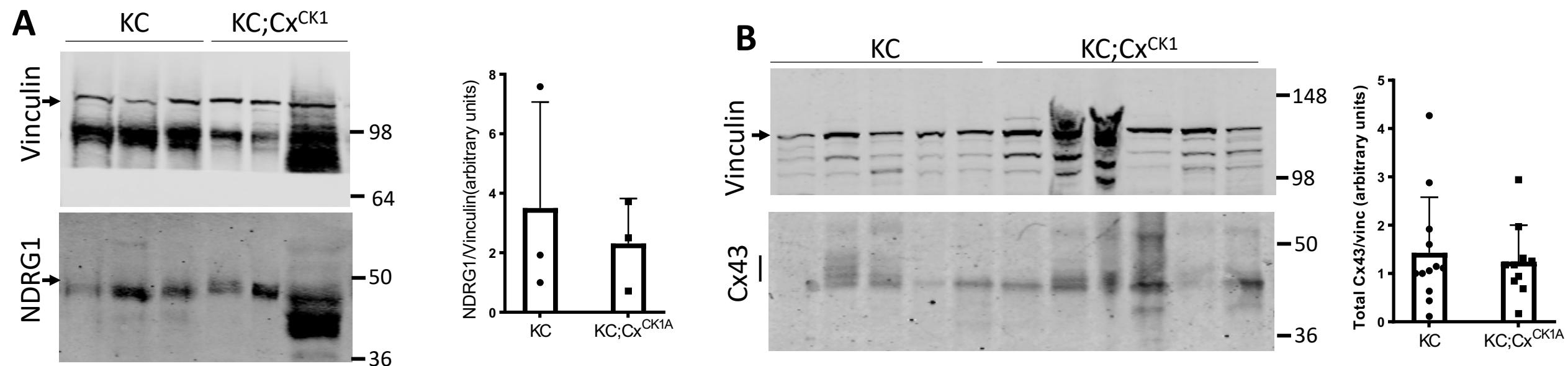
Representative immunofluorescent staining for PCNA (green) and DAPI (blue) in lungs from tail vein injection experiments. Number of PCNA positive nuclei were quantified for a subset of samples.

Supplemental Fig. 6 supports Fig. 3



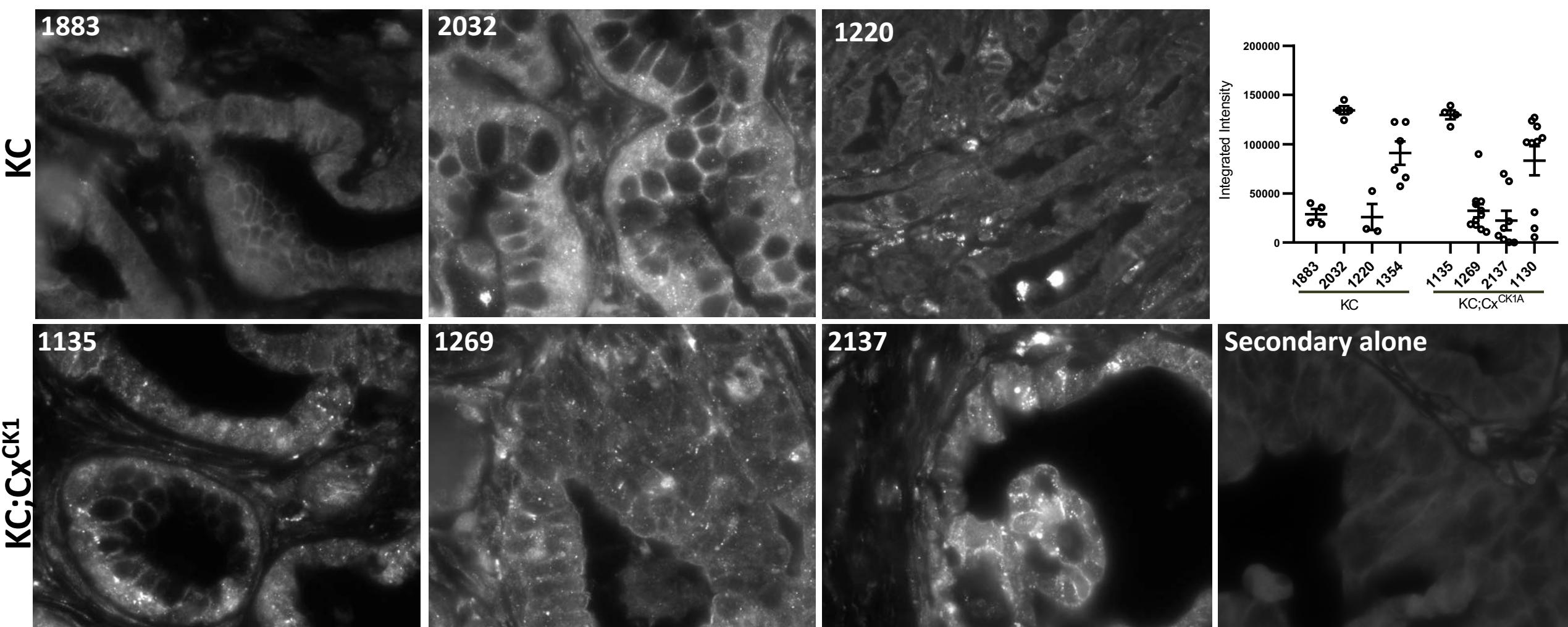
Immunoblot analysis of primary PDA tumor cell lines showing expression of Total ERK, phospho-ERK, Total Akt, phospho-Akt (pS308) and phospho-Akt (pT308).

Supplemental Figure 7 - supports Fig. 5



Immunoblot analysis of primary pancreatic tumor tissue. Graphs indicate densitometry measurements. A) NDRG1 and vinculin blots show lysates from 3 different KC and KC;Cx^{CK1} animals. Note this blot was cut just above the 64kD molecular weight marker to allow simultaneous blotting of NDRG1 and vinculin. B) Cx43 and vinculin blots show lysates from 5 different KC and 6 different KC;Cx^{CK1} animals

C NDRG expression in PDA tissue

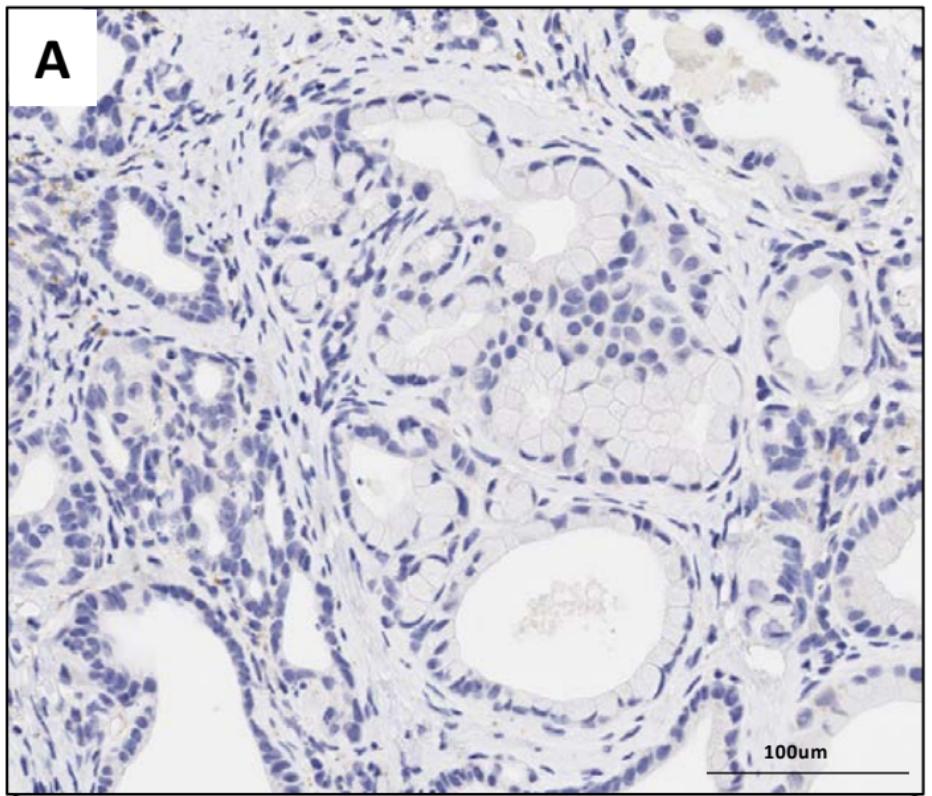


Immunofluorescent staining for NDRG1 on primary pancreatic tumor tissue from 3 different KC and KC;Cx^{CK1} mice. Secondary only panel shows a sample that did not receive primary antibody. Quantification of thresholded signal is shown in graph. Note a wide range of variability between animals regardless of genotype.

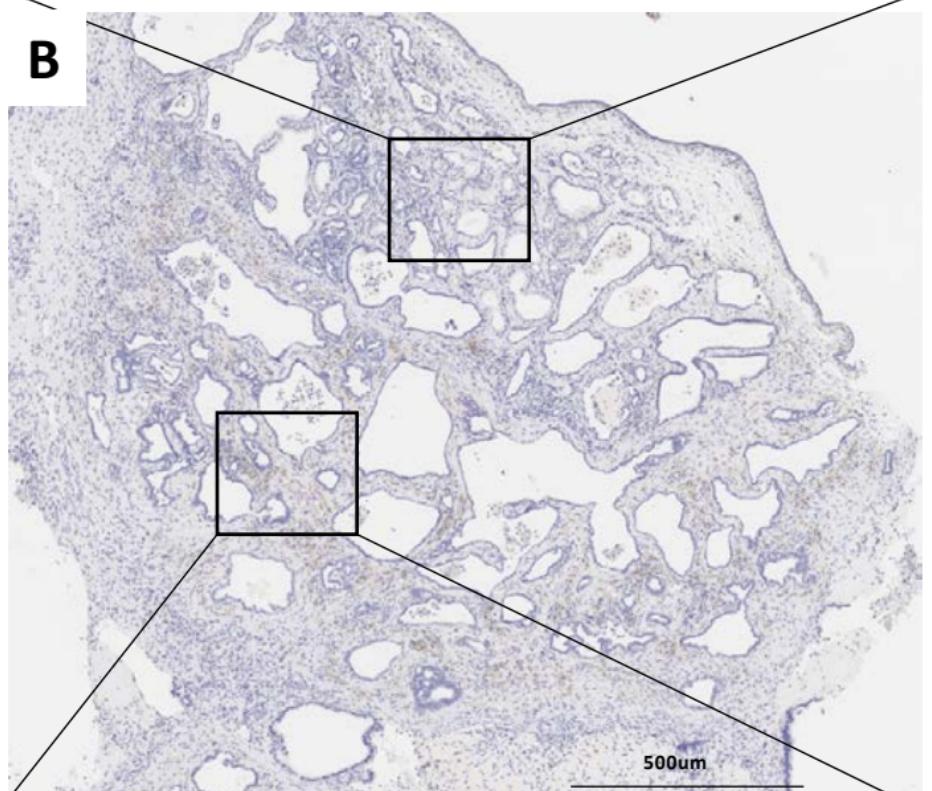
Supplemental Fig. 8

Supports Fig. 5

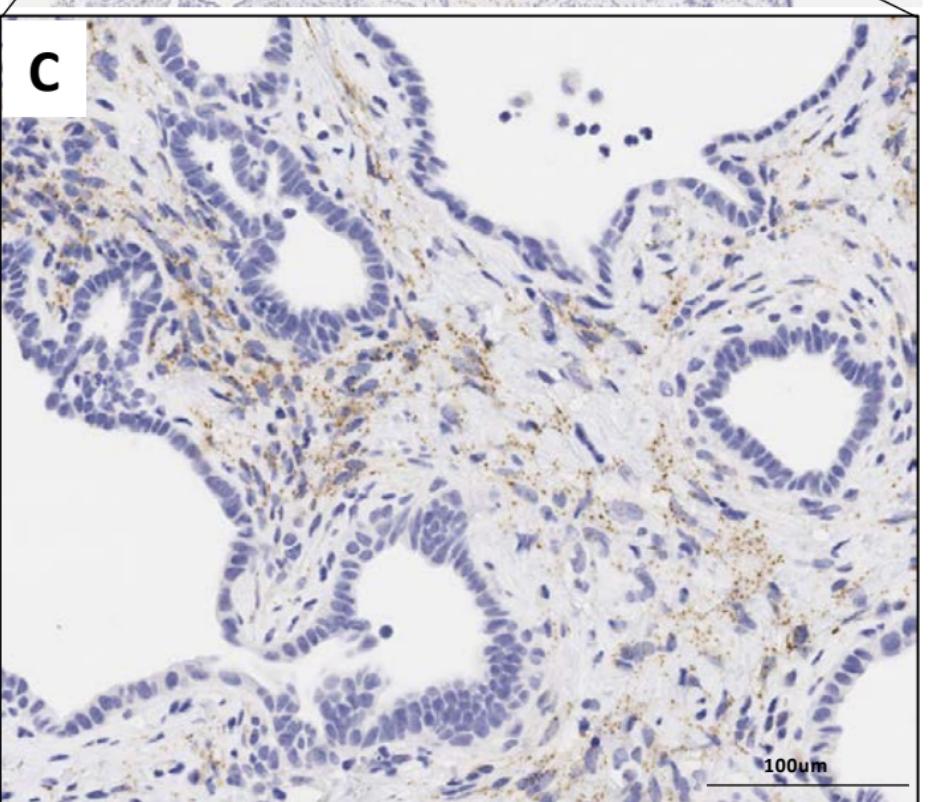
A



B

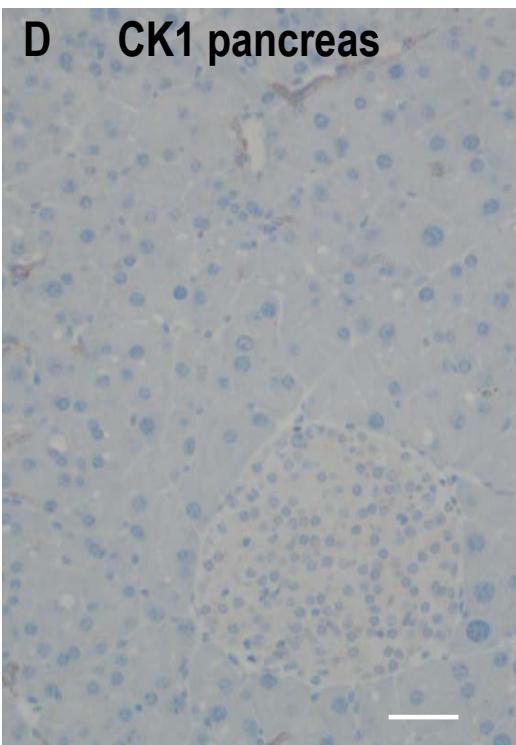
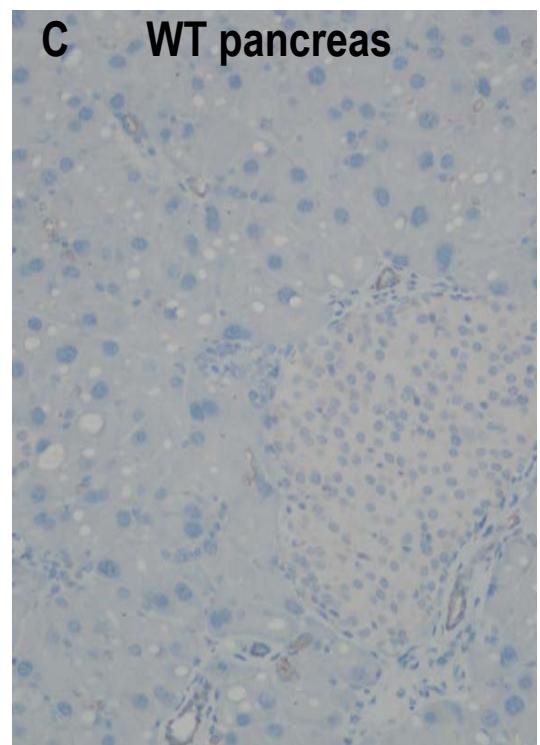
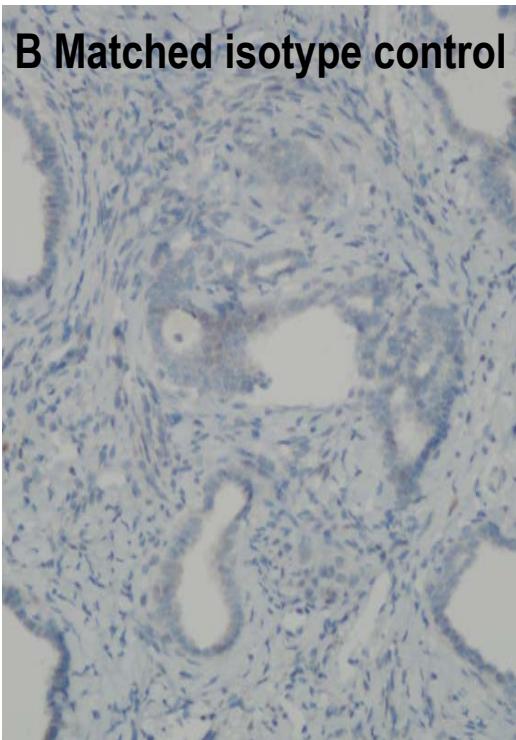
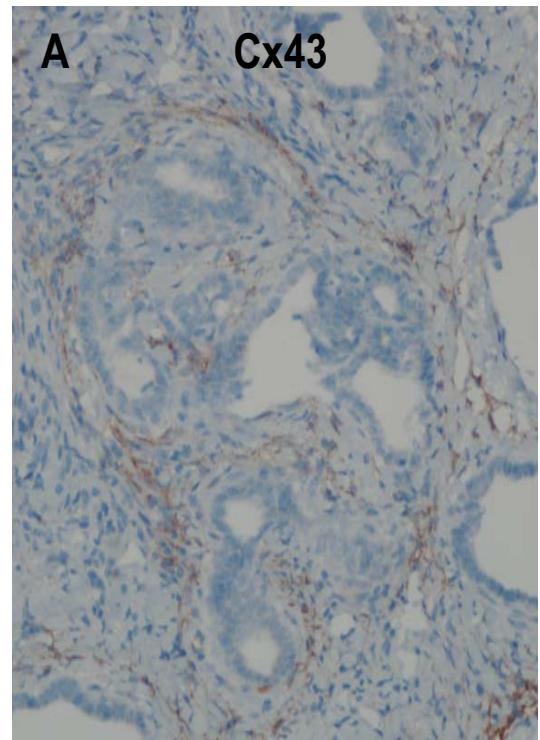


C



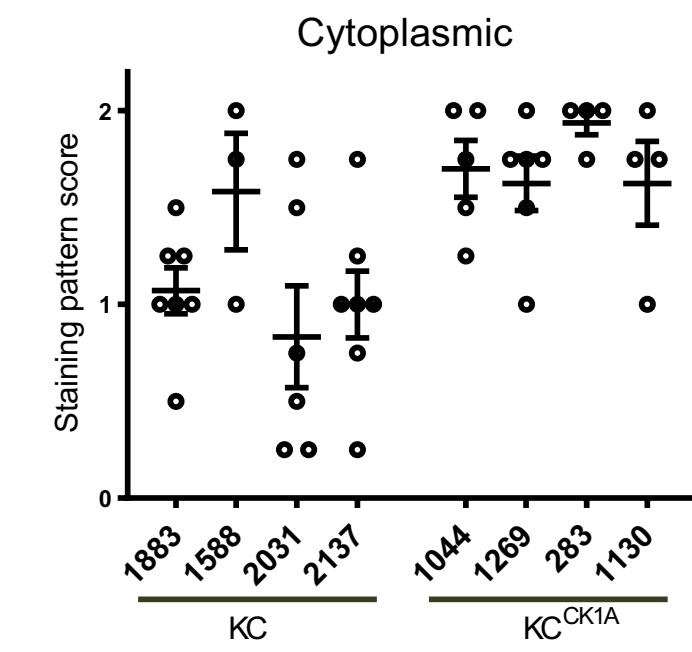
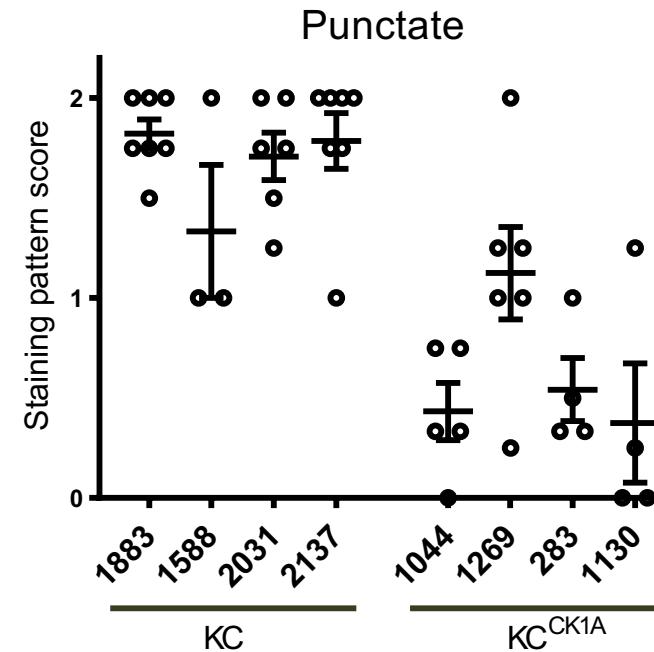
IHC staining for Cx43 in tissue from moribund KC mouse. B) shows low magnification while A) shows an area at high magnification with low Cx43 in the stroma and C) shows a nearby area with high Cx43 expression.

Supplemental Fig. 9 Supports Fig. 5



A. Cx43 staining of PDA tissue. B. Serial section showing isotype control. C. Cx43 staining of tissue from moribund WT mouse. D. Cx43 staining of tissue from moribund CK1 mouse. Bar=50 μ m.

E. Cx43 expression levels in individual mice



F. Ordinary one-way ANOVA p-values for individual mouse comparisons

	Punctate				ordinary one way ANOVA								
					KC;Cx ^{CK1A}						KC;Cx ^{CK1A}		
	KC		KC;Cx ^{CK1A}		1044	1269	283	1130	KC;KC	1269	1044	283	1130
KC	1883	<0.0001			0.001	<0.0001	<0.0001	<0.0001	KC;KC	1269	0.0003	0.0207	0.0003
	1588	<0.0001			0.9136		0.0021	<0.0001					
	2031	<0.0001			0.0207	<0.0001	<0.0001	<0.0001					
	2137	<0.0001			0.0024	<0.0001	<0.0001	<0.0001					
Cytoplasmic													
KC					KC;Cx ^{CK1A}						KC;Cx ^{CK1A}		
	1883	0.0095			0.0221		0.0009	0.0654	KC;KC	1588	0.2036	0.0109	0.0191
	1588	0.9994	>0.9999		0.8417	>0.9999							
	2031	<0.0001			0.0002	<0.0001	<0.0001	0.0015					
KC	2137	0.0004			0.001	<0.0001	<0.0001	0.0039					

E. Quantification of punctate and cytoplasmic staining for individual animals is shown.

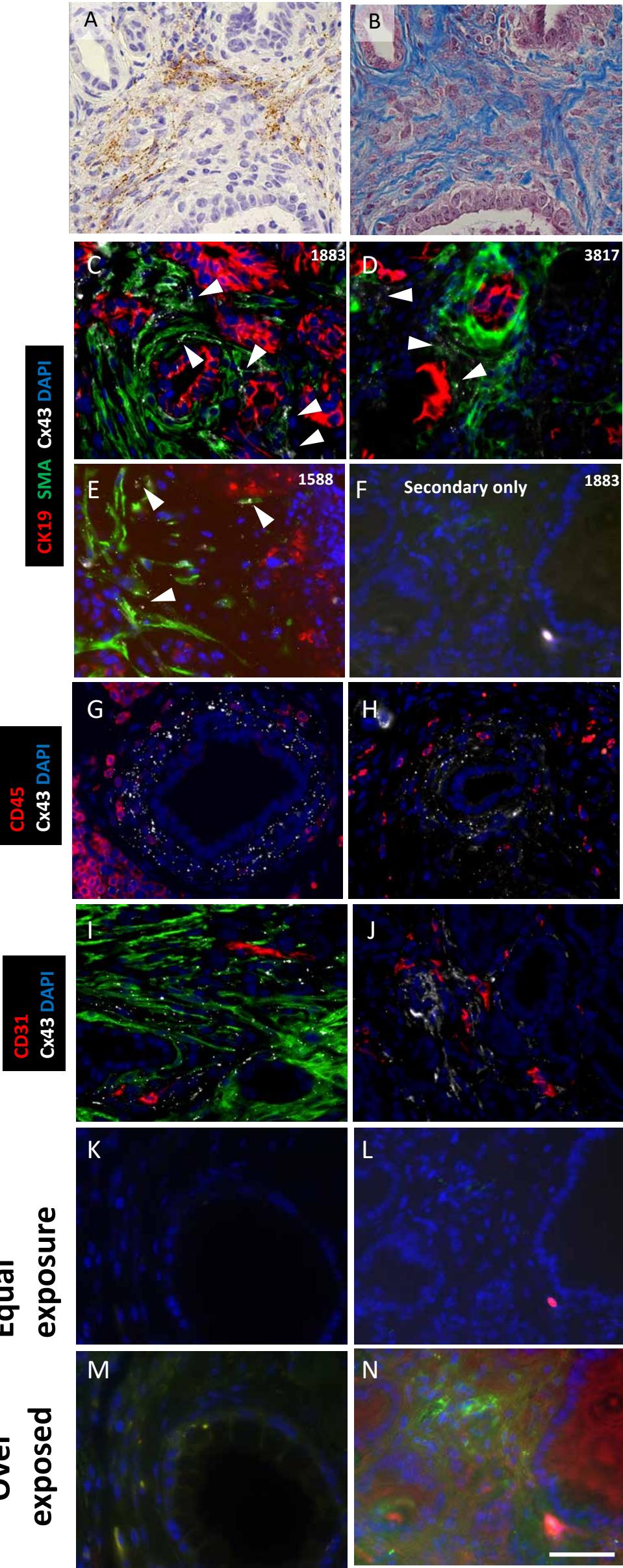
F. Table indicates p values (one-way ANOVA) for significant differences between animals. Non-significant values are noted in red.

Supplemental Fig. 10

supports Fig 6.

A) and B) Sequential sections of tissue from moribund KC mice stained for Cx43 and with Masson's Trichrome. C-E) Immunofluorescence of Cx43 (white), SMA (green) and CK19 (C, red) or CD31 (D, red). Arrowheads indicate gap junction staining. F) Secondary only panel shows an exposure 2 times longer than that used to collect images C-E. G-H) panels show Cx43 in white, CD45 in red, SMA in green and DAPI in blue. I-J) panels show CD31 in red, SMA in green and DAPI in blue. K and M) are secondary only controls for G) and I) respectively with image acquired under same conditions and L and N at longer acquisition times to highlight background staining. DAPI shown as blue. Bar is 50mm.

Equal exposure
Over exposed
Secondary only controls



Supplemental Fig. 11 supporting statistical analyses

	n		Statistical Test
	KC	KCCxCK1	
Survival study	23 (60%M, 40%F)	28 (44%M, 56%F)	Log Rank
Time course 6, 12 and 16m	10 (58%M,42%F)	10 (42%M,58%F)	Fisher's exact
Cyst incidence (16m cohort)	10	10	Fisher's exact
Classifier Acinar cells	6	6	unpaired t-test, two-tailed
Metastasis incidence	19	27	Fisher's exact
Image Quant. Alcian Blue	4	4	unpaired t-test, two-tailed
Classifier tail vein assay	3/group		ANOVA, Sidak's multiple comparisons
Cx43 expression tail vein assay	3/group		ANOVA, Sidak's multiple comparisons
Immunoblots	2	2	unpaired t-test, two-tailed
	Experiment repeated 4 different days		
Cx43 in moribund, punctate vs cytoplasmic	4	4	Nested t-test, 2-sided